EFFECTS OF DAURICINE AND TETRANDRINE ON [^{3}H] WEB 2086 SPECIFIC BINDING TO BOVINE ANTERIOR CEREBRAL ARTERIAL SMOOTH MUSCLE CELLS *IN VITRO*

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ABSTRACT By using [^{3}H] WEB 2086, a PAF antagonist, specific binding sites of PAF on bovine anterior cerebral arterial smooth muscle cells was identified. Two populations of binding sites with different dissociation constants on the cells were found. The K_{d-1} = 22.8±5.0 nmol·L^{-1}, K_{d-2} = 186±20.5 nmol·L^{-1} at 25 ℃. The total number of binding sites were B_{max-1} = 2.1±0.3 pmol/10^6 cells and B_{max-2} = 12.1±1.5 pmol/10^{6}cells. Dauricine and tetrandrine, two active compounds with similar chemical structure extracted from traditional Chinese herbs, were found to inhibit [^{3}H] WEB 2086 specific binding significantly in culture cells.

Key words [^{3}H] WEB 2086; Vascular smooth muscle; Receptor; PAF; Dauricine; Tetrandrine

Platelet activating factor (PAF) is a potent phospholipid mediator involved in inflammatory, cardiovascular and respiratory disorders. PAF receptors were reported to exist in brain[1], human polymorphonuclear leukocytes[2], eosinophils[3] and platelets[4,5]. In our previous studies, we found that PAF induced rabbit platelet aggregation and stimulated DNA synthesis and proliferation of bovine anterior cerebral arterial smooth muscle cells (BACASMC), and that dauricine and tetrandrine antagonized the effects of PAF on the above cells. We speculated that the effects of PAF on BACASMC might be receptor—mediated. In order to confirm this view, we in the present study used [^{3}H] WEB 2086, a PAF antagonist, to identify its specific binding sites on BACASMC. Also, the inhibitory effects of dauricine and tetrandrine on this kind of specific binding were studied.

MATERIALS AND METHODS

Reagents and drugs PAF was kindly provided by Dr. P. Hadvary (P. Hoffmann, La Roche,

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Swiss). [^{3}H] WEB 2086 with a specific activity of 51.8×10^{10} Bq·mmol^{-1} and WEB 2086 were gifts from Boehringer Ingelheim KG. Dauricine was obtained from the Department of Medicinal Chemistry, Chinese Pharmaceutical University, and tetrandrine was from our school.

Eagle's MEM was from Gibco Laboratory, USA. All MEM were supplemented with HEPES buffer 20 mmol·L^{-1}, penicillin 100 U·ml^{-1}, streptomycin 0.1 mg·ml^{-1} and 10% or 20% fetal calf serum (FCS), pH was adjusted to 7.2 with $NaHCO_3$.

Cell culture BACASMC were cultured as previously reported[6]. When the cells reached confluence, they were digested with 0.1% trypsin and suspended in reaction buffer containing Tris 10 mmol·L^{-1}, KCl 140 mmol·L^{-1} and 0.1% bovine serum albumin (BSA).

Binding assay BACASMC (3×10^5) were added to 0.5 ml of reaction buffer in glass tubes containing [^{3}H] WEB 2086 without or with 1000 fold unlabelled WEB 2086 (for nonspecific binding). The reaction was carried out at 25 C for 10 min. The unbound radioligand was separated from the bound by immediate filtration of the reaction solution through glass fiber presoaked in the reaction solution (without BSA) with a millipore filtration manifolding. Each filter was washed 5 times each with 2 ml of cold reaction buffer without BSA. The filter were air dried and placed into scintillation vials filled with 5 ml of scintillation fluid. The radioactivity was measured with a FJ—2107 liquid scintillation counter.

The [^{3}H] WEB 2086 specific binding was calculated by subtracting the nonspecific binding from the total. The binding data were analyzed by Scatchard plots.

RESULTS

Kinetic study

As shown in Fig 1, the specific binding of [^{3}H] WEB 2086 (8 nmol·L^{-1}) to BACASMC increased rapidly in the first 5 min and reached a steady state at 10 min and remained stable up to 30 min at 25 C. Nonspecific binding was found to be independent of time.

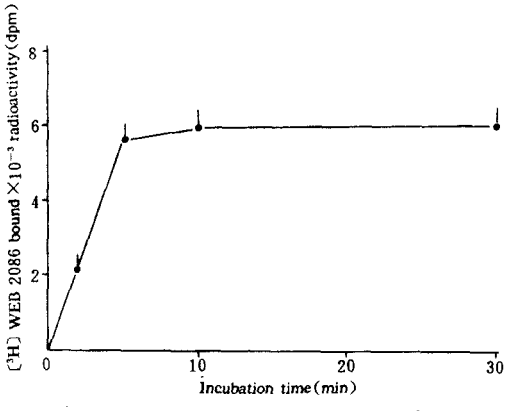

Fig 1 Kinetics of [^{3}H] WEB 2086 (8 nmol·L^{-1}) specific binding to bovine anterior cerebral arterial smooth muscle cells (3×10^5) at 25 C. $\bar{x}\pm s$, n=3.

Saturation study

The specific binding of [^{3}H] WEB 2086 was saturable and represented about 50% of the total binding at 40 nmol·L^{-1}, while nonspecific binding increased linearly with the radioligand concentrations. Scatchard analysis of the binding data indicates the existence of two classes of binding sites (Fig 2). The first population of binding sites presented a K_{d-1} = 22.8±5.0 nmol·L^{-1} and a total number of binding sites B_{max-1} = 2.1±0.3 pmol/10^6 cells, and the second one with a K_{d-2} = 186.1±20.5 nmol·L^{-1}, B_{max-2} = 12.1±1.5 pmol/10^6 cells.

Inhibition study

Fig 3 shows the displacement curves by WEB 2086, PAF, dauricine and tetrandrine. The maximal inhibitory rates of specific binding by these agents were 95.8, 72.3, 80.9 and 90.4% at 10^{-7}, 10^{-7}, 10^{-4} and 10^{-4} mol·L^{-1}, respectively.

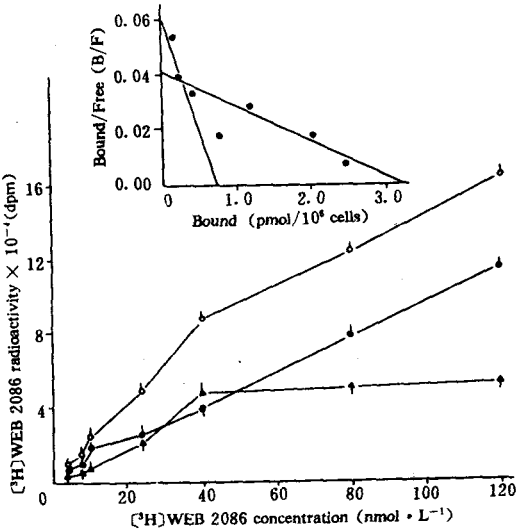

Fig 2 Saturation of [^{3}H] WEB 2086 specific binding to bovine anterior cerebral arterial smooth muscle cells. Total (○), nonspecific (•) and specific (▲) binding were measured at 25℃ for 10 min. $\bar{x}\pm s$, n = 3. (Inset) Scatchard analysis of [^{3}H] WEB 2086 specific binding to smooth muscle cells.

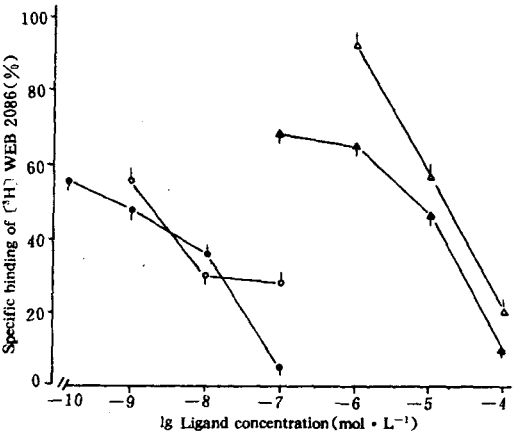

Fig 3 Inhibition of specific binding of [^{3}H] WEB 2086 (8 nmol·L^{-1}) to bovine anterior cerebral arterial smooth muscle cells by unlabelled WEB 2086 (•), PAF (○), dauricine (△) and tetrandrine (▲) at 25℃ for 10 min. $\bar{x}\pm s$, n = 3.

DISCUSSION

PAF targets are endothelial cells[7], platelets[4,5] etc. This study demonstrated the presence of PAF specific binding sites on BACASMC.

Scatchard analysis of the binding data showed the existence of two populations of binding sites on BACASMC. This is in accordance with other reports[1,2]. PAF was also found to promote DNA synthesis and TXA_2 production in BACASMC[8]. In view of our present study, however, it is speculated that these effects of PAF on BACASMC might be mediated by its specific receptors.

In this investigation, we also found that both dauricine and tetrandrine, two compounds from traditional Chinese herbs, exerted some inhibition on [^{3}H] WEB 2086 specific binding to BACASMC, and that tetrandrine was stronger. Our earlier study showed that both drugs inhibited the rabbit platelet aggregation induced by PAF, and tetrandrine was stronger. This is in coincidence with our present study, and indicates that both dauricine and tetrandrine might be effective in the prevention and treatment of cerebral vascular diseases.

REFERENCES

1 Domingo MT, et al. Presence of specific binding sites for platelet—activating factor (PAF) in brain. *Biochem Biophys Res Commun* 1988;**151**:730.

2 Marquis O, et al. [^{3}H] 52770RP, a platelet activating factor receptor antagonist, and tritiated platelet activating factor label a common specific binding site in human polymorphonuclear leukocytes. *J Pharmacol Exp Ther* 1988;**244**:709.

3 Ukena D, et al. PAF—receptors on eosinophils: identification with a novel ligand, [^{3}H] WEB 2086. *Biochem Pharmacol* 1989;**38**:1702.

4 Nunez D, et al. Inhibition of [^{3}H]platelet activating factor (PAF) binding by Zn^{2+}: a possible explanation for its specific PAF antiaggregating effects in human platelets. *Arch Biochem Biophys* 1989;**272**:146.

5 Janero DR, et al. Radioligand competitive binding methodology for the evaluation of platelet—activating factor (PAF) and PAF—receptor antagonism using intact canine platelets. *J Pharmacol Methods* 1988;**20**:237.

6 Zeng GQ, et al. The *in vitro* culture and morphological observation of bovine anterior cerebral arterial smooth muscle cells. *Acta Sec Mil Med Univ* 1991;**12**:167.

7 Bussolino F, et al. Human endothelial cells are target for platelet—activating factor. I. Platelet activating factor induces changes in cytoskeleton structures. *J Immunol* 1987;**139**:2439.

8 Zeng GQ and Rui YC. Effects of anisodamine and dauricine on proliferation, DNA synthesis, and calcium influx in bovine anterior cerebral arterial smooth muscle cells in culture. *Acta Pharmacol Sin* 1991;**12**:308.

蝙蝠葛碱和粉防己碱对[^{3}H] WEB 2086与体外牛脑前动脉平滑肌细胞结合的影响

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提要 用标记的血小板活化因子拮抗剂[^{3}H] WEB 2086，在培养的牛脑前动脉平滑肌细胞上鉴定了血小板活化因子受体。结果表明在25℃时该细胞上存在两种与配基具有不同亲和力的受体结合位点，其中 $K_{d-1}=22.8\pm5.0$ nmol·L^{-1}，$K_{d-2}=186\pm20.5$ nmol·L^{-1}；$B_{max-1}=2.1\pm0.3$ pmol/10^{6}细胞，$B_{max-2}=12.1\pm1.5$ pmol/10^{6}细胞。蝙蝠葛碱和粉防己碱均能抑制[^{3}H] WEB 2086与上述细胞的结合。

关键词 [^{3}H]WEB 2086；血管平滑肌；受体；血小板活化因子；蝙蝠葛碱；粉防己碱